GP 1646

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

17/c
2/12/03

In re Application of:
Miller et al.

Serial No: 09/670,049

Filed: September 25, 2000

For: Multipotent Neural Stem Cells from Peripheral Tissues and Uses Thereof

Attorney Docket No. CIBT-P03-120

Art Unit: 1646

Examiner: J. Murphy

CERTIFICATE OF MAILING UNDER 37 C.F.R. §1.8(a)

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Brent LaBarge

Commissioner of Patents
Washington, D.C. 20231

REPLY UNDER 37 CFR 1.111

Sir:

This amendment is being filed in reply to the outstanding Office Action mailed December 2, 2002, in connection with the above application. Please enter the following amendments:

In the claims:

For the convenience of the Examiner, all claims being examined, whether or not amended, are presented below.

Please cancel, without prejudice, claims 35, 45 and 46.

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18. (**Twice Amended**) A method of producing a population of at least ten cells, wherein at least 30% of the cells are multipotent stem cells substantially purified from epithelial tissue of a

18. **(Twice Amended)** A method of producing a population of at least ten cells, wherein at least 30% of the cells are multipotent stem cells substantially purified from epithelial tissue of a ~~postnatal mammal~~ human, or progeny of said multipotent stem cells, wherein said multipotent stem cells are self renewing, form non-adherent clusters, express nestin, and can differentiate into neuronal and mesodermal cell types, said method comprising the steps of:

(a) providing epithelial tissue from said human ~~mammal~~;

(b) culturing said epithelial tissue under conditions in which multipotent stem cells proliferate and in which at least 25% of the cells that are not multipotent stem cells die or attach to the culture substrate; and

(c) continuing culture step (b) until at least 30% of the cells are multipotent stem cells which are self renewing, form non-adherent clusters, express nestin, and can differentiate into neuronal and mesodermal cell types, or progeny of said multipotent stem cells.

19. **(Twice Amended)** A method of producing a population of at least ten cells, wherein at least 30% of the cells are multipotent stem cells substantially purified from epithelial tissue of a ~~postnatal mammal~~ human, or progeny of said multipotent stem cells, wherein said multipotent stem cells are self renewing, form non-adherent clusters, express nestin, and can differentiate into neuronal and mesodermal cell types, said method comprising the steps of:

(a) providing epithelial tissue from said human ~~mammal~~;

(b) culturing said epithelial tissue under conditions in which multipotent stem cells proliferate and in which at least 25% of the cells that are not multipotent stem cells die or attach to the culture substrate;

(c) separating said multipotent stem cells from said cells that attach to said culture substrate; and

(d) repeating steps (b) and (c) until at least 30% of the cells are multipotent stem cells which are self renewing, form non-adherent clusters, express nestin, and can differentiate into neuronal and mesodermal cell types, or progeny of said multipotent stem cells.

20. **(Reiterated)** The method of claim 19, wherein said population is at least one hundred cells.

36. **(New)** A method of producing a population of cells differentiated from multipotent stem cells, or progeny of said multipotent stem cells, comprising:

(a) obtaining an epithelial tissue from a mammal;

(b) culturing cells dissociated from said tissue under conditions wherein multipotent cells are expanded, which multipotent cells are characterized by the following: form non-adherent clusters in culture; are self renewing; express nestin; and differentiate into ectodermal and mesodermal cell types; and

(c) differentiating the multipotent cells into one or more lineage committed cell types.

37. **(New)** A method for preparing cell preparations, comprising:

(a) obtaining an epithelial tissue from a mammal;

(b) culturing cells dissociated from said tissue under conditions wherein multipotent cells are expanded, which multipotent cells are characterized by the following: form non-adherent clusters in culture; are self renewing; express nestin; and differentiate into ectodermal and mesodermal cell types; and

(c) differentiating the multipotent cells into one or more lineage committed cell types, wherein the conditions for differentiating the multipotent cells include plating on an adherent substratum.

38. **(New)** The method of claim 36 or 37, wherein the cells are formulated in a pharmaceutically acceptable carrier, auxiliary or excipient.

39. **(New)** The method of claim 36 or 37, wherein the lineage committed cells express one or more marker selected from the group consisting of Glial Fibrillary Acid Protein (GFAP), neurofilament 160, β III tubulin, NeuN, neurofilament-M (NFM), neuron-specific enolase, galactocerebroside, GAD, tyrosine hydroxylase (TH), dopamine β-dehydrogenase and CNPase.

40. **(New)** The method of claim 36 or 37, wherein the multipotent stem cells differentiate to at least one ectodermal cell type selected from the group consisting of astrocytes, oligodendrocytes, or neurons.

41. **(New)** The method of claim 40, wherein the neuron is a dopaminergic neuron.

42. **(New)** The method of claim 36 or 37, wherein the multipotent stem cells differentiate to at least one mesodermal cell type selected from the group consisting of skeletal muscle cells, cardiac muscle cells, connective tissue cells, lung cells, adipocytes, pancreatic islet cells, hematopoietic cells, chondrocytes, bone, and kidney cells.

43. **(Reiterated)** The method of claim 18 or 19, wherein said epithelial tissue is skin.

44. **(Reiterated)** The method of claim 18 or 19, wherein said epithelial tissue is tongue.

47. **(NEW)** A method of producing a population of at least ten cells, wherein at least 30% of the cells are multipotent stem cells substantially purified from skin or tongue tissue of a postnatal mammal, or progeny of said multipotent stem cells, wherein said multipotent stem cells are self renewing, form non-adherent clusters, express nestin, and can differentiate into neuronal and mesodermal cell types, said method comprising the steps of:

(a) providing skin or tongue tissue from said mammal;

(b) culturing said skin or tongue tissue under conditions in which multipotent stem cells proliferate and in which at least 25% of the cells that are not multipotent stem cells die or attach to the culture substrate; and

(c) continuing culture step (b) until at least 30% of the cells are multipotent stem cells which are self renewing, form non-adherent clusters, express nestin, and can differentiate into neuronal and mesodermal cell types, or progeny of said multipotent stem cells.

48. **(NEW)** A method of producing a population of at least ten cells, wherein at least 30% of the cells are multipotent stem cells substantially purified from skin or tongue tissue of a postnatal mammal, or progeny of said multipotent stem cells, wherein said multipotent stem cells are self renewing, form non-adherent clusters, express nestin, and can differentiate into neuronal and mesodermal cell types, said method comprising the steps of:

(a) providing skin or tongue tissue from said mammal;

(b) culturing said skin or tongue tissue under conditions in which multipotent stem cells proliferate and in which at least 25% of the cells that are not multipotent stem cells die or attach to the culture substrate;

(c) separating said multipotent stem cells from said cells that attach to said culture substrate; and

(d) repeating steps (b) and (c) until at least 30% of the cells are multipotent stem cells which are self renewing, form non-adherent clusters, express nestin, and can differentiate into neuronal and mesodermal cell types, or progeny of said multipotent stem cells

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18. (**Amended**) A method of producing a population of at least ten cells, wherein at least 30% of the cells are multipotent stem cells substantially purified from epithelial tissue of a postnatal mammal, or progeny of said multipotent stem cells, wherein said multipotent stem cells are self renewing, form non-adherent clusters, express nestin, and can differentiate into neuronal and mesodermal cell types, said method comprising the steps of:

(a) providing epithelial tissue from said mammal;

(b) culturing said epithelial tissue under conditions in which multipotent stem cells proliferate and in which at least 25% of the cells that are not multipotent stem cells die or attach to the culture substrate; and

(c) continuing culture step (b) until at least 30% of the cells are multipotent stem cells which are self renewing, form non-adherent clusters, express nestin, and can differentiate into neuronal and mesodermal cell types, or progeny of said multipotent stem cells.

19. (**Amended**) A method of producing a population of at least ten cells, wherein at least 30% of the cells are multipotent stem cells substantially purified from epithelial tissue of a postnatal mammal, or progeny of said multipotent stem cells, wherein said multipotent stem cells are self renewing, form non-adherent clusters, express nestin, and can differentiate into neuronal and mesodermal cell types, said method comprising the steps of:

(a) providing epithelial tissue from said mammal;

(b) culturing said epithelial tissue under conditions in which multipotent stem cells proliferate and in which at least 25% of the cells that are not multipotent stem cells die or attach to the culture substrate;

(c) separating said multipotent stem cells from said cells that attach to said culture substrate; and

(d) repeating steps (b) and (c) until at least 30% of the cells are multipotent stem cells which are self renewing, form non-adherent clusters, express nestin, and can differentiate into neuronal and mesodermal cell types, or progeny of said multipotent stem cells.

20. (**Reiterated**) The method of claim 19, wherein said population is at least one hundred cells.

Please add the following new claims:

35. (**New**) A method of producing a population of multipotent stem cells, or progeny of said multipotent stem cells, comprising:

(a) obtaining epithelial tissue from a mammal;

(b) culturing cells dissociated from said tissue; and

(c) isolating from the culture multipotent stem cells characterized by the following: form non-adherent clusters in culture; are self renewing; express nestin; and differentiate into ectodermal and mesodermal cell types.

36. (**New**) A method of producing a population of cells differentiated from multipotent stem cells, or progeny of said multipotent stem cells, comprising:

(a) obtaining an epithelial tissue from a mammal;

(b) culturing cells dissociated from said tissue under conditions wherein multipotent cells are expanded, which multipotent cells are characterized by the following: form non-adherent clusters in culture; are self renewing; express nestin; and differentiate into ectodermal and mesodermal cell types; and

(c) differentiating the multipotent cells into one or more lineage committed cell types.

37. (**New**) A method for preparing cell preparations, comprising:

(a) obtaining an epithelial tissue from a mammal;

(b) culturing cells dissociated from said tissue under conditions wherein multipotent cells are expanded, which multipotent cells are characterized by the following: form non-adherent clusters in culture; are self renewing; express nestin; and differentiate into ectodermal and mesodermal cell types; and

(c) differentiating the multipotent cells into one or more lineage committed cell types, wherein the conditions for differentiating the multipotent cells include plating on an adherent substratum.

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38. (**New**) The method of claim 36 or 37, wherein the cells are formulated in a pharmaceutically acceptable carrier, auxiliary or excipient.

39. (**New**) The method of claim 36 or 37, wherein the lineage committed cells express one or more marker selected from the group consisting of Glial Fibrillary Acid Protein (GFAP), neurofilament 160, β III tubulin, NeuN, neurofilament-M (NFM), neuron-specific enolase, galactocerebroside, GAD, tyrosine hydroxylase (TH), dopamine β-dehydrogenase and CNPase.

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43. (**New**) The method of claim 18 or 19, wherein said epithelial tissue is skin.

44. (**New**) The method of claim 18 or 19, wherein said epithelial tissue is tongue.

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45. (**New**) The method of claim 18 or 19, wherein said epithelial tissue is not olfactory epithelium.

46. (**New**) The method of claim 18 or 19, wherein said postnatal mammal is a human.

18. **(Amended)** A method of producing a population of at least ten cells, wherein at least 30% of the cells are multipotent stem cells substantially purified from epithelial tissue of a postnatal mammal, or progeny of said multipotent stem cells, said method comprising the steps of:

(a) providing epithelial tissue from said mammal;

(b) culturing said epithelial tissue under conditions in which multipotent stem cells proliferate and in which at least 25% of the cells that are not multipotent stem cells die or attach to the culture substrate; and

(c) continuing culture step (b) until at least 30% of the cells are multipotent stem cells or progeny of said multipotent stem cells.

19. **(Amended)** A method of producing a population of at least ten cells, wherein at least 30% of the cells are multipotent stem cells substantially purified from epithelial tissue of a postnatal mammal, or progeny of said multipotent stem cells, said method comprising the steps of:

(a) providing epithelial tissue from said mammal;

(b) culturing said epithelial tissue under conditions in which multipotent stem cells proliferate and in which at least 25% of the cells that are not multipotent stem cells die or attach to the culture substrate;

(c) separating said multipotent stem cells from said cells that attach to said culture substrate; and

(d) repeating steps (b) and (c) until at least 30% of the cells are multipotent stem cells or progeny of said multipotent stem cells.

20. **(Reiterated)** The method of claim 19, wherein said population is at least one hundred cells.

Please add the following new claims:

35. **(New)** A method of producing a population of multipotent stem cells, or progeny of said multipotent stem cells, comprising:

(a) obtaining epithelial tissue from a mammal;

(b) culturing cells dissociated from said tissue; and

(c) isolating from the culture multipotent stem cells characterized by the following: form non-adherent clusters in culture; are self renewing; express nestin; and differentiate into ectodermal and mesodermal cell types.

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(c) differentiating the multipotent cells into one or more lineage committed cell types.

37. **(New)** A method for preparing cell preparations, comprising:

(a) obtaining an epithelial tissue from a mammal;

(b) culturing cells dissociated from said tissue under conditions wherein multipotent cells are expanded, which multipotent cells are characterized by the following: form non-adherent clusters in culture; are self renewing; express nestin; and differentiate into ectodermal and mesodermal cell types; and

(c) differentiating the multipotent cells into one or more lineage committed cell types, wherein the conditions for differentiating the multipotent cells include plating on an adherent substratum.

38. **(New)** The method of claim 36 or 37, wherein the cells are formulated in a pharmaceutically acceptable carrier, auxiliary or excipient.

39. **(New)** The method of claim 36 or 37, wherein the lineage committed cells express one or more marker selected from the group consisting of Glial Fibrillary Acid Protein (GFAP), neurofilament 160, β III tubulin, NeuN, neurofilament-M (NFM), neuron-specific enolase, galactocerebroside, GAD, tyrosine hydroxylase (TH), dopamine β-dehydrogenase and CNPase.

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